DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
NTP Interagency Center for the Evaluation
of Alternative Toxicological Methods
P. O. Box 12233
Research Triangle Park, NC 27709
http://iccvam.niehs.nih.gov

June 26, 2009

Dr. Christine Augustyniak
U.S. National Coordinator for the
OECD Test Guidelines Program
U.S. Environmental Protection Agency
Ariel Rios Building, Mail Code 7509P
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Dear Dr. Augustyniak:

On behalf of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), we are pleased to provide a draft revision of OECD Test Guideline 429 *Skin Sensitisation: Local Lymph Node Assay* (LLNA), and two new draft test guidelines that describe non-radioactive versions of the LLNA, the LLNA: DA and the LLNA: BrdU-ELISA test methods. We are submitting these proposed draft test guidelines (TGs) pursuant to our submission of a Standard Project Submission Form (SPSF) to OECD in January 2008, and ask that you forward these expeditiously to the OECD Secretariat for consideration by the member countries.

The draft TGs for the LLNA: DA and the LLNA: BrdU-ELISA test methods were developed in conjunction with the Japanese Center for the Validation of Alternative Methods (JaCVAM), which sponsored multi-laboratory validation studies of these two test methods. The proposed TGs are based on draft ICCVAM recommended standardized test method protocols, which were developed based on an ICCVAM-sponsored international independent peer review panel evaluation of the validation status of each method (see 2009 Peer Review Panel Report at http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf#pagemode=bookmarks), comments from ICCVAM's advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods, and public comments received during the review process.

In addition to its evaluation of the two non-radioactive test methods, ICCVAM, in consultation with the European Centre for the Validation of Alternative Methods (ECVAM) and JaCVAM, developed performance standards for the LLNA (see ICCVAM Recommended Performance Standards: Murine Local Lymph Node Assay at

http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna-ps/LLNAPerfStds.pdf). These performance standards can be used to evaluate new specific protocol modifications (e.g., procedures to measure lymphocyte proliferation) that produce functionally and mechanistically similar variations of the traditional LLNA method. The performance standards are incorporated in the proposed update of the OECD Test Guideline 429. ICCVAM also evaluated the accuracy and reliability of a reduced LLNA (rLLNA) for hazard classification of skin sensitising substances, for use when dose-response information is not needed (see the ICCVAM Test Method Evaluation Report for the rLLNA at http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNA-LD/TMER.pdf). ICCVAM recommended that the rLLNA should be used for the hazard identification of skin sensitizing substances, if dose response information is not needed, provided there is adherence to all other LLNA protocol specifications. This recommendation has also been incorporated into the proposed update of OECD Test Guideline 429.

In testing situations where dose-response information is not required, ICCVAM also recommends that both the LLNA: DA and the LLNA: BrdU-ELISA should be considered for use as rLLNA protocols and that the ICCVAM-recommended performance standards should be used to evaluate any functionally and mechanistically similar variations of the LLNA: DA and the LLNA: BrdU-ELISA test methods. To facilitate the greatest reduction in animal use, ICCVAM proposes to incorporate these recommendations in the proposed test guidelines for these methods, to encourage the use of these non-radioactive LLNA methods by OECD member countries.

If it is determined that an Expert Consultation meeting is necessary based on the results of the commenting round by member countries, NICEATM-ICCVAM and the U.S. Consumer Product Safety Commission would be glad to organize and host such a meeting. We have reserved a meeting room at the CPSC Headquarters, 4330 East West Highway, Bethesda, Maryland, 20814, for October 20-22, 2009, and will provide all necessary administrative and logistical support for this meeting.

We believe that expeditious adoption of these proposed test guidelines will support further reduction and refinement of animal use for skin sensitization testing. Please feel free to contact us at any time if you have questions about the proposed test guidelines.

Sincerely,

/s/

William S. Stokes, D.V.M., D.A.C.L.A.M.
Rear Admiral, U.S. Public Health Service
Director, NTP Interagency Center for
the Evaluation of Alternative
Toxicological Methods (NICEATM)
National Institute of Environmental
Health Sciences

Marilyn L. Wind, Ph.D.
Chair, Interagency Coordinating
Committee on the Validation of
Alternative Methods (ICCVAM)
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety
Commission

Enclosures